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(71) Applicants *(for all designated States except US)*: CELLEGY PHARMACEUTICALS, INC. [US/US]; Suite 210, 371 Bel Marin Keys, Novato, CA 94949 (US). THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 22nd floor, 300 Lakeside Drive, Oakland, CA 94612-3550 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: ELIAS, Peter, M. [US/US]; Box 601, Star Route, Muir Beach, CA 94965 (US). THORNFELDT, Carl, R. [US/US]; 1054 N.W. 2nd Avenue, Ontario, Oregon 97914 (US). GRAYSON, Stephen [US/US]; 27 Mt. Foraker Drive, San Rafael, CA 94903 (US).

(74) Agents: WETHERELL, John, R., Jr. et al.; Spensley Horn Jubas & Lubitz, 1880 Century Park East, Fifth floor, Los Angeles, CA 90067 (US).

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(54) Title: METHOD AND COMPOSITIONS FOR DISRUPTING THE EPITHELIAL BARRIER FUNCTION

(57) Abstract

A method for disrupting epithelial barrier function in a host in need of the topical administration of a physiologically active substance which comprises applying to the epithelium of the host, barrier-disrupting amount of at least one agent selected from the group consisting of an inhibitor of ceramide synthesis, inhibitor of acylceramide synthesis, inhibitor of glucosylceramide synthesis, and inhibitor of sphingomyelin synthesis, an inhibitor of fatty acid synthesis, an inhibitor of cholesterol synthesis, a degradation enzyme of ceramides, acylceramide, glucosylceramides, sphingomyelin, an inhibitor of phospholipid, glycosphingolipid, including glucosylceramide, acylceramide or sphingomyelin degradation, and both inhibitors and stimulators of metabolic enzymes of free fatty acids, ceramide, and cholesterol, as well as a topical composition useful therefor are disclosed.

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METHOD AND COMPOSITIONS FOR DISRUPTING THE EPITHELIAL BARRIER FUNCTION

This is a Continuation-in-Part application of U.S. Serial No. 08/033,811, filed March 19, 1993.

BACKGROUND OF THE INVENTION

1. *Field of the Invention*

This invention relates generally to a novel method for enhancing penetration of physiologically active substances for cutaneous or transdermal delivery through epithelium which comprises the stratum corneum/epidermis and keratinizing mucous membranes. More specifically, it relates to a method and composition for disrupting the epithelial barrier function in a host which employs at least one agent selected from the group consisting of inhibitors of ceramide synthesis, an inhibitor of glucosylceramide synthesis, an inhibitor of acylceramide synthesis, an inhibitor of sphingomyelin synthesis, an inhibitor of fatty acid synthesis, an inhibitor of cholesterol synthesis, inhibitors of phospholipid, glycosphingolipid, including glucosylceramide, acylceramide and sphingomyelin degradation, a degradation enzyme of free fatty acid, ceramide, acylceramide, or glucosylceramides and sphingomyelin, and both inhibitors and stimulators of metabolic enzymes of free fatty acids, ceramide, and cholesterol.

2. *Description of Related Art*

The major function of the epithelium is that of a barrier to prevent the excessive loss of bodily fluids. If this barrier is disrupted or perturbed, it stimulates a variety of metabolic changes in the epithelium leading to repair of the barrier defect. While the barrier protects against external damage induced by such agents as ultraviolet radiation, desiccation, chemicals, and frictional or blunt

trauma, it impedes the pen tration of topically applied medicaments, nutrients, or other x nobiotics.

The epithelial barrier is a system of multilayered membrane lipid bilayers that exist throughout the intercellular spaces of the stratum corneum in epidermis and keratinizing mucous membranes. The bilayers in stratum corneum of epidermis consist of approximately equimolar ratios of three major lipid species: ceramides, free fatty acids, and cholesterol, as well as small, but critical, amounts of acylceramides. Keratinizing mucous membrane multilayered bilayers consist of approximately equimolar ratios of glucosylceramides, free fatty acids and cholesterol. These lipid species are synthesized in the subjacent nucleated cell layers of the epithelium. Following any type of barrier perturbation, an increase in lipid biosynthesis occurs, which leads to the recovery of barrier structure and function. The more extensive the perturbation of the barrier, the more active is the subsequent lipid biosynthetic response.

In addition to the long-standing approaches of hydration and occlusion, currently available percutaneous and transmucosal penetration enhancement technology relies on physical-chemical methods, such as solvents or detergents, and physical approaches, such as iontophoresis, electroporation, or sonophoresis. Typical solvents or detergents alter the physical properties of the multilayered lipid bilayers. Such agents include dimethylsulfoxide (DMSO), oleyl alcohol (OA), propylene glycol (PG), methyl pyrrolidone and AZONE® (dodecylazyl cycloheptan 2-one). For example, U.S. Patent No. 4,177,267 discloses topical steroid compositions containing dimethylsulfoxide as an epithelial penetration enhancer. It is generally believed that many of these epithelial penetration enhancers fluidize the polar head group (e.g., DMSO) and/or nonpolar tail group (e.g., OA) domains within the multilayered lipid bilayers. Yet, some compounds with significant fluidizing effect have been shown to be incapable of substantially increasing epithelial permeability. While

th s methods nhance penetration of certain compounds by three- to five-fold, th s methods ar only relatively effective for smaller lipophilic and amphiphathic molecules. Hydrophilic compounds such as proteins or peptides do not penetrate in pharmaceutically useful quantities through the epithelia even when most of these permeation technologies are utilized.

Accordingly, there is a need for epithelial penetration enhancers capable of allowing and/or increasing the penetration of large and/or water-soluble molecules in therapeutically effective quantities. This invention addresses this need by providing methods and topical compositions for disrupting the epithelial barrier thereby facilitating the penetration of therapeutic known or potential molecules.

SUMMARY OF THE INVENTION

It has been discovered that a formulation comprising at least one agent selected from the group consisting of inhibitors of ceramide synthesis, an inhibitor of glucosylceramide synthesis, an inhibitor of acylceramide synthesis, an inhibitor of fatty acid synthesis, an inhibitor of cholesterol synthesis, inhibitors of phospholipid, glycosphingolipid, acylceramide and sphingomyelin degradation, is very effective for disrupting epithelial barrier function in a host, and thereby enhancing penetration of a physiologically active substance administered topically.

In one aspect thereof, this invention provides a method for disrupting epithelial barrier function in a host in need of topical administration of a physiologically active substance which comprises applying to the skin of the host a barrier-disrupting amount of at least one agent selected from the group consisting of an inhibitor of ceramide synthesis, an inhibitor of acylceramide synthesis, an inhibitor of glucosylceramide synthesis, an inhibitor of free fatty acid synthesis, an inhibitor of cholesterol synthesis, an inhibitor of phospholipid or glycosphingolipid, acylceramide and sphingomyelin degradation, both inhibitor and a stimulator of steps of free fatty acid ceramide and cholesterol metabolism distal to these compounds, and a degradation enzyme of free fatty acids, ceramides, acylceramide, and glucosylceramides or sphingomyelin or glucosylceramides and both inhibitors and stimulators of metabolic enzymes of free fatty acids, ceramide, and cholesterol.

In another aspect, this invention provides a topical composition for disrupting epithelial barrier function in a host in need of topical administration of a physiologically active substance which comprises an epithelial barrier-disrupting amount of at least one agent selected from the group consisting of an inhibitor of ceramide synthesis, an inhibitor of acylceramide synthesis, an

inhibitor of glucosylceramid synth sis, an inhibitor of sphingomyelin synthesis, an inhibitor of free fatty acid synth sis, and an inhibitor of cholesterol synthesis, inhibitors of phospholipid, glycosphingolipid, including glycosylceramide, acylceramide and sphingomyelin degradation, a degradation enzyme of free fatty acid, ceramide, acylceramide, sphingomyelin or glucosylceramides and both inhibitors and stimulators of metabolic enzymes of free fatty acids, ceramide, and cholesterol

The above features and advantages of this invention will be more fully understood by reference to the following detailed description and the drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 schematically shows a biosynthetic and degradation pathway of ceramides, acylceramides and glucosylceramides.

FIGURE 2 schematically shows a biosynthetic pathway of free fatty acids.

FIGURE 3 schematically shows a biosynthetic and degradation pathway of cholesterol.

FIGURE 4A shows the propylene glycol concentrations at an epidermal surface of control animals when they were treated with a vehicle. ○, VBL; ○, VBR; □, VCL; ■, VCR.

FIGURE 4B shows the propylene glycol concentrations at an epidermal surface of tested animals when they were treated with fluvastatin (fluindostatin). ○, FBL; ○, FBR; □, FCL; ■, FCR.

FIGURE 5A shows the cyclophenol concentrations at an epidermal surface of control animals when they were treated with a vehicle. ○, VBL; ○, VBR; □, VCL; ■, VCR.

FIGURE 5B shows the cyclophenol concentrations at an epidermal surface of tested animals when they were treated with fluindostatin. ○, FBL; ○, FBR; □, FCL; ■, FCR.

FIGURE 6A shows TEWL when animals were treated with oleic acid followed by fluindostatin.

FIGURE 6B shows TEWL when animals were treated with oleic acid followed by 5-(tetradecyloxy)-2-furoic acid (TOFA).

DETAILED DESCRIPTION OF THE INVENTION

This invention is based on the discovery that when the biosynthesis of one or more of the epithelial lipids, ceramides, acylceramide, glucosylceramides, sphingomyelin, cholesterol and free fatty acids is inhibited, or their distal utilization is increased, the lipid species is depleted perturbing the normal mole ratio, resulting in incompetent epithelial barrier function. Inhibition of enzymes involved in a biosynthetic pathway or inhibition of the degradation enzymes for the precursors of each of these key lipid constituents have also been specifically targeted according to the present invention. Further, it has been discovered that the inhibition of biosynthetic enzymes or stimulation of degradative enzymes aiming at two or more lipid constituents may be additive or synergistic in the opening of the epithelial barrier for percutaneous or transmucosal delivery of physiologically active substances.

The composition of this invention principally employs an epithelial barrier-disrupting amount of at least one agent selected from the group consisting of inhibitors of ceramide synthesis, an inhibitor of sphingomyelin synthesis, an inhibitor of glucosylceramide synthesis, an inhibitor of acylceramide synthesis, an inhibitor of sphingomyelin synthesis, an inhibitor of fatty acid synthesis, an inhibitor of cholesterol synthesis, inhibitors of phospholipid, glycosphingolipid, including glucosylceramide, acylceramide and sphingomyelin degradation, a degradation enzyme of free fatty acid, ceramide, acylceramide, or glucosyl-ceramides and sphingomyelin and both inhibitors and stimulators of metabolic enzymes of free fatty acids, ceramide, and cholesterol.

As used herein, the term "an epithelial barrier-disrupting amount" means that the amount of enzyme inhibitor(s), stimulator(s) or degradation enzymes are of sufficient quantity to disrupt the barrier when these compounds are topically applied to the skin or mucous membrane of a host. The amount can vary

according to the effectiveness of each enzyme inhibitor or stimulator, or degradation enzyme, as a percutaneous or transmucosal penetration enhancer, the host age, and response of the host. More importantly, the therapeutic amount should be determined based on the penetration efficiency of a given physiologically active substance when that substance is administered in conjunction with a particular combination of the enzyme inhibitors or stimulators, or degradation enzymes. The required quantity to be employed in this invention can be determined readily by those skilled in the art.

As used herein, the term "host" includes humans and non-human mammals. Non-human mammals of particular interest are domesticated species such as dogs, cats, monkeys, cows, horses, llamas, sheep, pigs, and goats.

The term "penetration enhancement" or "permeation enhancement" as used herein relates to an increase in the permeability of skin to a physiologically active substance, i.e., so as to increase the rate at which the substance permeates through the epithelium and enters the bloodstream.

As applied in this invention, the term "physiologically active substance" is intended to encompass any substance that will produce a physiological response when topically administered to a host. In general, the terms include therapeutic or prophylactic agents in all major therapeutic/prophylactic areas of medicine as well as nutrients, cofactors, enzymes (endogenous or foreign), antioxidants or other defensive principals, and xenobiotics. Suitable substances include, but are not restricted to, antifungals such as amphotericin B, griseofulvin, miconazole, ketoconazole, tioconazol, itraconazole, and fluconazole; antibacterials such as penicillins, cephalosporins, tetracyclines, aminoglucosides, erythromicin, gentamicins, polymyxin B; anti-cancer agents such as 5-fluorouracil, bleomycin, methotrexate, hydroxyurea; anti-inflammatories such as hydrocortisone, glucocorticoids, colchicine, ibuprofen,

indomethacin, and piroxicam; antioxidants, such as tocopherols, retinoids, carotenoids, ubiquinones, metal chelators, and phytic acid; antihypert nsive agents such as prazosin, verapamil, nifedipine, and diltiazem; analgesics such as acetaminophen and aspirin; anti-viral agents such as acyclovir, ribavarin, and trifluorothyridine; antiandrogens such as spironolactone; androgens such as testosterone; estrogens such as estradiol; progestins such as modified progestogens; opiates; muscle relaxants such as papaverine; vasodilators such as nitroglycerin, vasoactive intestinal peptide and calcitonin related gene peptide; antihistamines such as cyproheptadine; agents with histamine receptor site blocking such as doxepin, imipramine, and cimetidine; antitussives such as dextromethorphan; neuroleptics such as clozaril; antiarrhythmics; antiepileptics; and other polypeptides and neuropeptides such as somatostatin, various cytokines, insulin, gastrin, substance P, and capsaicin; and enzymes, such as superoxide dismutase and neuroenkephalinase. Other useful drugs, in approved commercially available formulations, and their recommended dosages are listed in the annual publication of the Physicians' Desk Reference, published by Medical Economics Company, a division of Litton Industries, Inc. More than one physiologically active substance may be included, if desired, in the topical composition of this invention.

The active substance may be water-soluble or water-insoluble and may include higher molecular weight proteins, peptides, carbohydrates, glycoproteins, lipids, and glycolipids. Such proteinaceous active substances which can be included are immunomodulators and other biological response modifiers. Examples of immune response modifiers include such compounds as cytokines, including tumor necrosis factors, interleukins, growth factors, colony stimulating factors, and interferons.

The active substance will be present in the composition in an amount sufficient to provide the desired physiological effect with no apparent toxicity to the host.

Of course, the appropriate dosage levels of all the physiologically active substances, without the use of the epithelial barrier-disrupting agents of the present invention, are known to one skilled in the art. These conventional dosage levels correspond to the upper range of dosage levels for compositions, including a physiologically active substance and an epithelial barrier-disrupting agent. However, because the delivery of the active substance is enhanced by the epithelial barrier-disrupting agent of this invention, dosage levels significantly lowering the conventional dosage levels may be used with success. In general, the active substance will be present in the composition in an amount of from about 0.0001% to about 60%, more preferably about 0.01% to about 20% by weight of the total composition depending upon the particular substance employed.

Ceramides, including acylceramides, account for 40-50% by weight of the stratum corneum lipids while glucosylceramides account for a similar percent in mucosal membranes. Representative *in vivo* biosynthetic pathways for these lipid species are shown in Fig. 1. Among many enzymes involved in these biosynthetic pathways, serine palmitoyl transferase is the rate limiting enzyme.

The inhibitors of ceramide, acylceramide, sphingomyelin, and glucosylceramide synthesis and metabolism include inhibitors of serine palmitoyl transferase such as β-chloroalanine, fluoropalmitate, and β-fluoroalanine, and inhibitors of ceramide synthetase such as fumonisins. Inhibitors of sphingomyelinase include agents such as tricyclodecan-9-yl-xanthogenate, ethylisopropylamiloride, N-palmitoyl-DL-dihydrosphingosine, methylene-dioxybenzapine, tricyclodecan-9yl-xanthogenate; aminoglycosides including gentamicin and neomycin; ethyliso-proplylamiloride; tricyclic antidepressants, including desipramine and imipramine; and phenothiazines including chlorpromazine and perchlorperazine. Inhibitors of glucosylceramide synthesis further include inhibitors of UDP-glucose-ceramide glucosyl transferase (glucosyl transferase), such as 1-phenyl-

2-deanoylamino-3-morphalino-1-propanol (PDMP), its analogs, α- and β-xylosides; and alpha xylosides including p-nitro-phenyl-α-xyloside and beta xylosides including 4-methyl umbelliferyl-β-0-xyloside, 0-and p-nitrophenyl-β-0-xylopyaranoside. Inhibitors of acylceramide synthesis further include inhibitors of α-hydroxylation, N-acyl chain length elongation, and ω-acyl transferase. Inhibitors of acid lipase include the boronic acids, including phenylboronic acid, tetrahydrolipstatin and esterasin.

In keratinizing mucous membrane, glycosphingolipids, including glucosylceramide, are not metabolized into ceramide, as occurs in the stratum corneum. Therefore, inhibitors of glycosphingolipids including inhibitors of β-glucosidase such as N-hexylglucosyl-sphingosine, bromoconduritol B-epoxide, conduritol, cyclophellitol, conduritol B-epoxide, and deoxynojirimycin will effectively perturb the barrier in the stratum corneum, but not in the keratinized mucous membranes.

D-cycloserine, β-chloroalanine, L-cycloserine, β-fluoroalanine, fluoropalmitate, and fumonisins are preferred inhibitors of epithelial sphingolipid synthesis, with β-chloroalanine and fumonisins being most preferred. Stimulators of glucosyl transferase, α-hydroxylation, N-acyl chain length elongation, and ω-acyl transferase and phosphotidylcholine-ceramide phosphorylcholine transferase will effect epithelial ceramide and glucosylceramide concentrations. An effective concentration range for these inhibitors and stimulators in the topical composition of this invention is generally from about 0.0001% to about 20% by weight of the total, with about 0.01% to about 5% preferred.

Free fatty acids account for 20-25% of the epithelial lipids by weight. The free fatty acids are synthesized and metabolized *in vivo* as shown in Fig. 2. The two rate limiting enzymes in the biosynthesis of the free fatty acids are acetyl

CoA carboxylas and fatty acid synthetase. Through a series of steps, free fatty acids are metabolized into phospholipids.

The inhibitors of free fatty acid synthesis and metabolism include inhibitors of acetyl CoA carboxylase such as 5-tetradecyloxy-2-furancarboxylic acid (TOFA); inhibitors of fatty acid synthetase; inhibitors of phospholipase A such as gomisin A, 2-(p-amylcinnamyl)amino-4-chlorobenzoic acid, bromophenacyl bromide, monoalide, 7,7-dimethyl-5,8-eicosadienoic acid, nicergoline, cepharanthine, quercetin, dibutyryl-cyclic AMP, R-24571, N-oleoylethanolamine, N-(7-nitro-2,1,3-benzoxadiazol-4-yl) phosphostidyl serine, cyclosporine A, topical anesthetics, including dibucaine, prenylamine, retinoids, such as all-trans and 13-cis-retinoic acid, W-7, trifluoperazine, R-24571 (calmidazolium), 1-hexadocyl-3-trifluoroethyl glycero-sn-2-phosphomenthol (MJ33); calcium channel blockers including verapamil, diltiazem, nifedipine, and nimodipine; antimalarials including quinacrine, mepacrine, chloroquine and hydroxychloroquine; beta blockers including propanalol and labetalol; calmodulin antagonists; EGTA; thimersol; glucocorticosteroids including dexamethasone and prednisolone; and nonsteroidal antiinflammatory agents including indomethacin and naproxen. TOFA is the preferred inhibitor of free fatty acid synthesis. An effective concentration range for the fatty acid inhibitor in the topical composition of this invention is generally from about 0.0001% to about 20% by weight of the total, with a preferred range of about 0.01% to about 5%.

Free sterols, primarily cholesterol, account for 20-25% of the epithelial lipids by weight. The free sterols are synthesized and metabolized *in vivo* as shown in Fig. 3. The rate limiting enzymes in the biosynthesis of cholesterol is 3-hydroxy-3-methylglutaryl (HMG) CoA reductase.

The inhibitors of cholesterol synthesis include competitive inhibitors of (HMG) CoA reductase such as simvastatin, lovastatin, fluindostatin (fluvastatin),

pravastatin, mevastatin, as well as other HMG CoA reductase inhibitors, such as cholesterol sulfate and phosphate, and oxyg nated sterols, such as 25-OH- and 26-OH-cholest rol; inhibitors of squalene synthetase; inhibitors of squalene epoxidase; inhibitors of $\Delta 7$ or $\Delta 24$ reductases such as 22,25-diazacholesterol, 20,25-diazacholestenol, AY9944, and triparanol. The preferred inhibitors are fluindostatin, simvastatin, lovastatin, cholesterol sulfate, and 25-OH-cholesterol. An effective concentration range for the cholesterol inhibitor in the topical composition of this invention is generally from about 0.0001% to about 20% by weight of the total, with a preferred range of about 0.01% to about 5%.

The degradation enzyme of ceramide is ceramidase. The degradation enzymes of acylceramides are acid lipase followed by ceramidase. The degradation enzymes of glucosylceramide are β-glucocerebrosidase followed by ceramidase. The degradation enzyme of sphingomyelin is sphingomyelinase. An inhibitor of ceramidase is N-oleoyl-ethanolamine. An effective concentration range for these degradation enzymes is generally from about 0.0001% to about 20% by weight of the total, with a preferred range of about 0.01% to about 5%.

The term "stimulators of steps of ceramide, free fatty acid, and cholesterol metabolism distal to these molecules" means molecules capable of shunting cholesterol, free fatty acid or cholesterol to more distal metabolites, such as glucosylceramide, acylceramide, sphingomyelin, phospholipids; and steroid hormones, respectively. This has the effect of depleting free fatty acid, cholesterol, or ceramides. An effective concentration range for such stimulators is generally from about 0.0001% to about 20% by weight of the total, with a preferred range of about 0.01% to about 5%.

Several of the metabolic pathways mentioned herein have as yet no known inhibitors for the enzymes, such as alpha-hydroxylation, N-acyl chain length

elongation, omega-acyl transferases, phosphatidylcholine-ceramide phosphoryl-choline transferases, fatty acid synthetase, squalene synthetase, cholesterol phosphate synthetase, cholesterol sulfate synthetase. However, should such inhibitors be discovered, the use of such inhibitors is contemplated within the scope of this invention.

These enzyme inhibitors, degradation enzymes, or enzyme stimulators can be co-applied to the skin or mucous membrane of a host in a formulation with any combination of these compounds with or without conventional penetration enhancers or other drug delivery technology, including transdermal patches, iontophoretic and electrophoretic devices, and sonicators. Alternatively, they can be applied concurrently as separate formulations. Still further, one agent can be applied before, simultaneously with, or after application of the other agent(s) provided that the time interval between the two (or more) is not too lengthy (e.g, typically, not more than about 24 hours). The physiologically active substance can be co-administered to the host with a topical composition which contains these inhibitors or stimulators. Alternatively, the pharmacologically active substance may be administered after application of the topical composition of the invention. It is, however, preferred to use the enzyme inhibitors or stimulators, or degradation enzymes, with the pharmacologically active compound as a single composition or formulation.

Preferably and conveniently, the combined or single inhibitor is applied to the skin in combination with a physiologically acceptable carrier. The carrier may comprise any conventional topical formulation base such as those described in Remington's "Pharmaceutical Sciences," 17th Edition (Mack Publishing Co., Pa), the disclosure of which is incorporated by reference. A lotion, solution, cream, ointment, paste, gel, suppository, aerosol, or nebulized formulation are representative of the topical compositions of this invention.

Additional ingredients may be added to the topical composition, as long as th y are physiologically acceptable and not deleterious to the epithelial cells and function. Such additives should not adversely affect the epithelial penetration efficiency of the above-noted enzyme inhibitors or stimulators, or degradation enzymes, nor cause the stability of the composition to deteriorate. Examples of ingredients which can be added to the compositions of the invention include stabilizers, preservatives, buffering agents, surfactants, emulsifiers, fragrances, humectants, and the like.

In one embodiment of this invention, a known percutaneous penetration-enhancing compound may be included in the composition to be additive or synergistic with the above enzyme inhibitors or stimulators, or degradation enzymes. Some of such penetration-enhancing compounds are described in U.S. Patent Nos. 4,424,210 and 4,316,893, the disclosures of which are incorporated by reference. The preferred compounds include propylene glycol, methyl pyrrolidone, oleyl alcohol, DMSO and AZONE®. The use level of the additional penetration-enhancing compounds is not significantly different from that of the enzyme inhibitors or stimulators, or degradation enzymes, and is in the range of from about 0.0001% to about 20.0% and preferably about 0.01% to about 5.0% by weight of the topical composition.

Topical lovastatin, an HMG CoA reductase inhibitor, is shown to impair the recovery of barrier function, assessed as transepidermal water loss (K.R. Feingold, *et al., J. Clin. Invest.* 88, 1338-1345, 1991). Also, β-chloro-L-alanine, an irreversible inhibitor of serine-palmitoyl-transferase, is shown to slow barrier recovery, assessed as transepidermal water loss (W.M. Holleran, *et al., J. Clin. Invest.* 88:1338, 1991). However, these references neither teach nor suggest that either compound is capable of disrupting epidermal barrier function sufficient for percutaneous drug delivery. It is recognized by one skilled in the art that the inhibition of barrier recovery to excess water loss (inside to outside)

and the disruption of epidermal barrier function sufficient for delivery of molecules much larger than water from the outside to the inside are not correlated.

The effectiveness of the topical compositions of this invention to enhance penetration of a physiologically active substance at an epithelial site can be determined by their ability to disrupt the normal diffusion profile of marker compounds such as cyclophenol or propylene glycol through the skin.

While the present invention has been described with respect to preferred embodiments thereof, it will be understood that various changes and modifications will be apparent to those skilled in the art and that it is intended that the invention encompass such changes and modifications as falling within the scope of the appended claims. The following non-limiting examples are provided to further illustrate the present invention.

EXAMPLE 1

The following ingredients were combined and blended uniformly together to produce a gel formulation:

INGREDIENTS	PERCENT BY WEIGHT
Fluindostatin	2.0
β-chloroalanine	1.5
Carboxyvinyl polymer 940	1.0
Ethanol	30.0
Propylene glycol	30.0
Triethylamine	1.5
Distilled water	Remaining part

A solution was prepared by mixing all the ingredients except triethylamine. Neutralization of the aqueous solution with triethylamine furnished a viscous gel.

Pharmaceutically active substances such as hydroxycortisone can be added to this gel for anti-inflammatory therapy.

EXAMPLE 2

The following ingredients were combined and blended uniformly together to produce an ointment formulation:

INGREDIENTS	PERCENT BY WEIGHT
Fluindostatin	1.5
β-chloroalanine	1.0
Plastibase 50W (mineral oil 95%, polyethylene 5%)	Remaining part

Blends of the active ingredients in ointment base were mixed together for 30 minutes at 40 rpm followed by 60 minutes at 25 rpm under vacuum to prevent aeration.

Pharmaceutically active substances such as erythromicin can be added to this ointment for antibacterial therapy.

EXAMPLE 3

The following ingredients were combined and blended uniformly together to produce a cream formulation:

INGREDIENTS	PERCENT BY WEIGHT
TOFA	1.5
Fluindostatin	1.0
β-chloroalanine	1.0
Cetyl/stearyl alcohol	25.0
Glycerin	5.0
Oleic acid oleyl ester	3.0
Distilled water	Remaining part

Cetyl/stearyl alcohol (25 g), 10 g of an aqueous suspension of the active ingredient and 3 g of oleic acid oleyl ester were heated to 80°C and emulsified by stirring at that temperature with a mixture of 5 g of glycerin and 57 ml of water.

Pharmaceutically active substance such as ketoconazole can be added to this cream for antifungal therapy.

EXAMPLE 4

The following ingredients were combined and blended uniformly together to produce a cream formulation:

INGREDIENTS	PERCENT BY WEIGHT
TOFA	1.0
Cetyl/stearyl alcohol	40.0
Polysorbate 80	10.0
Distilled water	Remaining part

Pharmaceutically active substance such as nifedipine can be added to this cream for antihypertensive therapy.

EXAMPLE 5

EPIDERMAL BARRIER DISRUPTION

To determine the epidermal barrier disruption by application of fluindostatin, an animal testing was conducted in the manner as follows:

Hairless mice (two animals, B and C) were topically pretreated with fluindostatin for 7 days. In a control group, animals (two) were only treated with a vehicle. The vehicle used was a mixture of propylene glycol (PG) and ethanol (0.5 ml of 5% w/v per deuterated PC in ethanol). The parameters for the vehicle in all of the experiments herein are as follows: TEWL 266 ± 22 (T=4 hours); plasma concentration 0.31 ± 0.03%; total body concentration 13.13 ± 0.83 (% dose/ml plasma); N=14.

After treatment, the skin surface was cleaned and stratum corneum allowed to recover to its normal state of hydration for 2 hours. An IR spectrum of the hydrophilic marker compound, propylene glycol, was recorded, and a single tape-stripping was conducted at the treated skin site. Another spectrum was recorded followed by the removal of a second tape strip. The same sequence was repeated 10 times. Figs. 4A, 4B show the test results. In Fig. 4A, control data indicate a gradual decrease of the PG concentration, while the data in Fig. 4B show lower absolute levels of PG in the stratum corneum, and a constant concentration level of PG, indicative of loss of the diffusional barrier to drug penetration. These results demonstrate that there is an absolute reduction of the permeability function and loss of the diffusion gradient with fluindostatin treatment, resulting in enhanced percutaneous transport of the marker compound, propylene glycol.

In essentially the same manner, cyclophenol (CP) was used as a lipophilic marker. Tape-stripping was repeated 7 times after application of fluindostatin.

Test results ar shown in Figs. 5A, 5B. In Fig. 5A, control data indicate a gradual decrease of the CP concentration, whil the data in Fig. 5B show a lower, constant concentration level of CP.

Both sets of data demonstrate that fluindostatin caused significant disruption of the stratum corneum barrier, and enhanced drug delivery as illustrated both by loss of the diffusion gradient, and by the lower absolute concentrations of the marker compounds in the stratum corneum.

EXAMPLE 6

TEWL (TRANS-EPIDERMAL WATER LOSS)

A female hairless mouse, aged 8 weeks, was treated with oleic acid. After oleic acid treatment, fluindostatin and/or 5-tetradecyloxy-2-furancarboxylic acid (TOFA) was applied to the animal.

TEWL (trans-epidermal water loss) was measured before and after treatment at convenient time intervals when the animal was alert. An Evaporimeter (ServoMed) was used.

First, TEWL of the left (LHS) and right (RHS) dorsal sides was measured. Then, the animal was anesthetized by 0.25 ml chloral hydrate (CH) IP injection. At t=0, an aliquot of 30 μl oleic acid solution (5% in propylene glycol/ethanol 7:3 (v/v)) was applied directly to both sides of the animal (~1.5 x 3 cm) using a Hamilton syringe. Two hours after oleic acid treatment, the TEWL rates of both sides were measured, and the RHS was treated with 30 μl of fluindostatin (1.5% fluindostatin in propylene glycol/ethanol 7:3 (v/v)) or TOFA (0.25% TOFA in propylene glycol/ethanol 7:3 (v/v)). TEWL was measured thereafter every 2 or 3 hours.

Test results are shown in Figs. 6A and 6B. These data demonstrate that both fluindostatin and TOFA delay the recovery of the barrier, after prior barrier disruption by the oleic acid treatment.

EXAMPLE 7
TEWL (TRANS-EPIDERMAL WATER LOSS) CORRELATION WITH DRUG DELIVERY

To prove that disruption of the stratum corneum permeability barrier by this invention results in successful delivery of known pharmacologically active compounds the plasma and total body concentrations of lidocaine and luteinizing hormone releasing hormone (LHRH) after topical application, since all pharmaceuticals do not have the same physicol chemical characteristics of these two tested compounds, TEWL was measured also using the following protocol.

Four hairless mice were anesthetized with chloral hydrate as described in Example 6 and treated with acetone to disrupt the lipid barrier until the desired TEWL level was attained as monitored by a electrolytic water analyzer (Meeco Inc., Warrington, PA). The mice were kept under anesthesia until the harvest of tissue samples and then sacrificed. At time zero, the tested drug delivery compound listed in Tables 2 - 7 below was applied to the whole treated flank of each mouse. After four hours TEWL was measured again and two drug formulations, one containing lidocaine and the other containing Luteinizing Hormone Releasing Hormone (LHRH) in a vehicle of 60% ethanol, 20% propylene glycol and 20% water. Residual formulation was removed from the skin surface with cotton balls three times and the cotton balls were put into vial #1. Five tape-strippings were conducted at the treated skin sites, and each tape was put into an individual vial numbered 2-6. At the same time, blood was drawn and stored under refrigeration. Urine was collected from the mice during the two hour drug application period and placed into vial #8.

After an additional two hours, the treated skin was cut off, the subcutaneous fat was removed, and the whole skin was placed into vial #7 to which 1 ml of

tissue solubilizer was added, and the mixture was allowed to digest ov rnight at 55°C. The corpse was digested in 100 ml of saponification mix at 55°C overnight.

For analysis, 10 ml of Scintisafe (30%) Scintillation media (Fisher Scientific, Fairlawn, NJ) was added to vials 1-6, and 8. The blood samples were centrifuged, and an aliquot of 100-200 μl was placed into vial #9 and 10 ml of Scintisafe (30%) was added. Similar aliquots of the corpse digest were placed into vials #10 and #11. To vials #9, 10, and 11, 100 μl of 30% hydrogen peroxide was added to decolorize the contents for about two hours. Then 16 μl of Scintisafe (30%) and 150 μl of acetic acid were added and the mixture was let stand overnight. All vials were subjected to scintillation counting as described above.

By testing pairs of inhibitors or stimulators, each from a different metabolic pathway, faster screening could be achieved. Individual compounds within successful pairs were then tested to confirm barrier disruption and increased drug delivery activity of the sole compound. Two compounds from each chemical class were tested. The inhibitors and metabolic pathways are listed in Table 1 below. The vehicle for these studies is an effective drug delivery agent in its own right comprising propylene glycol ethanol, and water. The vehicle parameters are: TEWL 266 ± 22 (T=4 hours); plasma concentration 0.31 ± 0.03%; total body concentration 13.13 ± 0.83 (% dose/ml plasma); N=14.

A portion of the LHRH plasma concentration specimens have been analyzed with a radioimmunoassay (RIA) at Hazelton, Washington Laboratories, Vienna, Virginia to determine if successful transdermal delivery of this peptide occurred with Cellegy's drug delivery technology. Unfortunately, the LHRH RIA is a new test that seems to have a relatively higher amount of errors than more

established RIA for other pharmacologically activ compounds. The LHRH uptakes are listed in Table 8. The numbers reflect the ratio of the plasma levels following application of the tested drug delivery compound(s) to the vehicle, which is a very good delivery system itself. The wide range of variability (indicated by SEM) in the results does not allow for establishment of p values.

TABLE 1

Inhibitor	Code	Inhibited Enzyme	Critical Lipid Metabolic Pathway
5-Tetradecyloxy-2-furancarboxylic acid	TOFA	Acetyl CoA Carboxylase	Free Fatty Acid
Fluindostatin	FLU	HMG CoA Reductase	Cholesterol
Lovastatin	LOV	"	"
Cholesterol sulfate	CS	"	"
β-Chloroalanine	BCA	Serine Palmitoyl Transferase	Ceramide
L-Cycloserine	LCS	"	"
Fumonisin B_1	FUM	Ceramide Synthetase	"
1-Hexadecyl-3-trifluoroethyl glycero-sn-2-phosphomenthol	MJ33	Phospho-lipase A_2	Free Fatty Acid, Ceramide
Bromophenacylbromide	BPB	"	"
Conduritol-β-epoxide	CBE	Beta- glucocere-brosidase	Ceramide
Deoxynojirimycin	DOJ	"	"
Desipramine	DIN	Sphingo-myelinase	"
N-Palmitoyl-DL-dihydroxysphingosine	NPHS	"	"
N-Oleoylethanolamine	NOEA	Ceramidase, Phospho-lipase A_2	Ceramide, Free Fatty Acid
4-Methylumbelliferyl-β-o-xyloside	MBX	Glucosyl-transferase	Ceramide Degradation
Acid lipase	ALP	Degredation	Acylceramide Degredation
1-Phenyl-2-decanoylamino-3-morpholino-1-propanol	PDMP	Glucosyl-transferase	Ceramide Degradation
2-[n-morpholino]ethanesulfonic acid	MES	Buffer	All

EXAMPLE 8

Studies were conducted using th same protocols as in Example 7 to inhibit rate-limiting synthetic enzymes for the three lipids that are critical for stratum corneum barrier function -- free fatty acid, ceramide, and cholesterol. Inhibition disrupts the critical mole ratio of 1:1:1, thereby perturbing barrier function. Acetyl CoA Carboxylase (ACC) is the rate-limiting synthetic enzyme for free fatty acids, and its only known inhibitor is 5-tetradecyloxy-2-furancarboxylic acid (TOFA). Serine palmitoyl transferase (SPT) is the rate-limiting synthetic enzyme for ceramide, and its chemically unrelated inhibitors betachloroalanine and L-cycloserine were evaluated. 3-hydroxy-3-methylglutaryl (HMG) CoA Reductase is the rate-limiting synthetic enzyme for cholesterol and is inhibited by three compounds that were tested - fluindostatin and lovastatin, which are chemically related, and cholesterol sulfate.

When the seven combinations of any two of these inhibitors of the critical lipid rate-limiting enzymes were applied together, they produced a statistically very significant ($p \leq 0.001$) increased delivery of lidocaine. Five combinations very significantly increased stratum corneum water permeabilities measured by TEWL and shown in Table 2. The TOFA combined with lovastatin or L-cycloserine produced a statistically significant increased water permeability, ($p=0.002$-0.05). TOFA combined with betachloroalanine produced the greatest delivery of lidocaine in this assay by increasing the drug's delivery by eight fold over vehicle control.

The TOFA and lovastatin combination successfully delivered the peptide LHRH transdermally as evidenced by plasma levels being 1.9 times higher than the amount of vehicle delivered as shown in Table 8.

EXAMPLE 9

Using the same protocol as in Example 8, the critical lipid rate-limiting enzyme inhibitors were tested as solitary agents as shown in Table 3. TOFA, fluindostatin, and cholesterol sulfate as the solitary inhibitors each produced a statistically very significant increase in lidocaine delivery while beta chloroalanine produced a significant increase. TOFA very significantly increased water permeability while fluindostatin and cholesterol sulfate significantly increased it. Beta chloroalanine did not increase stratum corneum water permeability in this assay.

EXAMPLE 10

In this study, the same protocols as used in Example 9 above were employed to test the effect upon transdermal delivery of lidocaine and LHRH by a combination of a rate-limiting enzyme inhibitor used in Example 9 with a nonrate-limiting synthetic enzyme of another of the three critical lipids. As shown in Table 4 below, all seven of these combinations successfully delivered lidocaine, with five producing a very significant increase. Fumonisin B1 combined with TOFA or fluindostatin, significantly increased lidocaine delivery.

Conduritol B epoxide, an inhibitor of β glucocerebrosidase, applied with fluindostatin very significantly increased stratum corneum water permeability but when combined with TOFA a significant increase in water permeability resulted.

Fumonisin B1, an inhibitor of ceramide synthetase, when combined with cholesterol sulfate, very significantly increased water permeability. When fumonisin B1 was applied with TOFA a significant increase of water permeability resulted.

When N-palmitoyl-DL-dihydroxysphingosine (NPHS), an inhibitor of sphingomyelinase, was combined with fluindostatin, water permeability was significantly increased.

TOFA combined with N-palmitoyl-DL-dihydroxysphingosine (NPHS) successfully delivered transdermally the peptide LHRH to a level of 6.9 times higher than the amount delivered by the vehicle as shown in Table 8.

EXAMPLE 11

When two nonrate-limiting enzymes from different critical lipid biosynthetic pathways are combined, statistically very significant increased delivery of lidocaine occurs, as shown in Table 5. Seven of the eight tested combinations produced very significant or significant increases in stratum corneum water permeability also.

Inhibitors of phospholipase A such as bromophenacylbromide (BPB) and MJ33 are particularly active because they actually inhibit both free fatty acid and ceramide synthesis. All eight of the tested combinations comprise one of these two inhibitors. BPB combined with conduritol B epoxide, each at low concentration, produced a statistically significant increase in water permeability and a nearly significant (p=0.05-0.10) increase in lidocaine delivery. When the concentrations of these two inhibitors were increased by 5 and 4 fold respectively, water permeability and lidocaine delivery were both very significantly increased. When bromophenacylbromide is applied with desipramine, a sphingomyelinase inhibitor, a very significant increase of lidocaine and water permeability occurred at low concentration, but when desipramine was doubled only lidocaine delivery was very significant. This data suggests a dose response curve occurs with this invention and that the active agents do not act as non-specific destructive agents to the barrier.

When fumonisin B1 is applied with either BPB or MJ33, a very significant increase in lidocaine delivery, with a significant increase in water permeability occurs. When BPB is applied with deoxynojirimycin, an inhibitor of B glucocerebrosidase, or NPHS, very significant increases of lidocaine delivery and water permeability resulted.

BPB combined with NPHS most successfully delivered LHRH by increasing this p ptide's delivery by 7.6 times greater than the amount delivered by the vehicle as shown in Table 8. BPB combined with deoxynojirimycin, and the combination of fumonisin B1 and MJ33 each increased delivery of LHRH by 2.0 and 1.7 times respectively as compared to vehicle.

Therefore, deficiency of free fatty acid and ceramide disrupts the critical lipid ratio, thereby perturbing the stratum corneum barrier and allowing increased drug delivery and water permeability.

EXAMPLE 12

When th non rate limiting enzyme inhibitors were used as solitary agents, usually the results were favorable, as shown in Table 6. The importance of inhibitor concentration as mentioned in Example 11 above is shown by conduritol B epoxide alone at a single application failing even nearly significantly to increase either water permeability or lidocaine delivery, even when the skin was prepermeabilized with acetone. Yet when it was frequently applied, even to intact skin, statistically significant increased lidocaine delivery and water permeability resulted.

The importance of intact skin application compared to prepermeabilized skin is demonstrated by the tested results of BPB. As a solitary agent, it nearly significantly increased lidocaine delivery, but not water permeability. But when applied to prepermeabilized skin, very significant increases of both lidocaine delivery and water permeability resulted.

Fumonisin B1 and N-oleolyethanolamine, an inhibitor of both phospholidase A and ceranidase, each very significantly increased lidocaine delivery while NPHS significantly increased it. Both NPHS and N-oleolyethanolamine very significantly increased water permeability. Fumonisin B1 successfully delivered LHRH as shown in Table 8 by increasing its delivery by 3.8 times greater than the vehicles.

EXAMPLE 13

The critical mole ratio of the three lipids in the stratum corneum barrier is also disrupted if normal degradation does not occur due to metabolic enzyme inhibition resulting in critical lipid accumulation as shown in Table 7. This concept was proven when lidocaine was successfully delivered and water permeability increased when ceramide metabolic pathways were blocked, resulting in its accumulation. The combination of N-oleoylethanolamine, with either PDMP or 4-methylumbelliferyl-B-O-xyloside, both inhibitors of glucosyl transferase, produced a statistically very significant increase in lidocaine delivery. The latter combination also significantly increased water permeability.

Acid lipase, metabolizes acylceramide to ceramide. Its application also results in significantly increased lidocaine delivery and water permeability.

Acid lipase as a solitary delivery compound increased LHRH plasma concentration by 2.2 times as compared with its vehicle, Morpholinoethane Sulfonic Acid (MES), as shown in Table 8.

TABLE 2

Compounds	N	Conc (%)	TEWL (T=4 hours)	P	Plasma Conc. (%dose /ml plasma)	P	Total Body Conc. (%)	P
TOFA + fluindostatin	4	0.5/1.5	475 ± 34	0.001	0.78 ± 0.11	<.001	35.62 ± 7.17	<.001
TOFA + cholesterol sulfate	4	0.5/1.0	588 ± 54	<.001	1.52 ± 0.35	<.001	69.03 ± .68	<.001
TOFA + β-chloroalanine	4	0.5/1.0	520 ± 1	<.001	2.47 ± 0.22	<.001	52.22 ± 3.90	<.001
TOFA + lovastatin	4	0.5/2.5	440 ± 79	0.014	1.35 ± 0.34	<.001	48.23 ± 7.87	<.001
β-chloroalanine + cholesterol sulfate	4	1.0/1.0	525 ± 47	<.001	0.68 ± 0.10	<.001	37.28 ± 4.46	<.001
Fluindostatin + β-chloroalanine	4	1.5/1.0	513 ± 41	<.001	0.48 ± 0.06	0.018	25.75 ± 2.78	<.001
TOFA + L-cycloserine	4	0.5/1.0	553 ± 56	0.021	1.32 ± 0.29	<.0001	32.3 ± 3.17	<.0001

TABLE 3

Compounds	N	Conc (%)	TEWL (T=4 hours)	P	Plasma Conc. (%dose /ml plasma)	P	Total Body Conc. (%)	P
TOFA	4	1.0	520 ± 31	<.001	1.20 ± 0.16	<.001	39.69 ± 6.49	<.001
Fluindostatin	4	1.5	431 ± 46	0.004	0.57 ± 0.19	0.133	23.69 ± 7.10	0.001
β-chloroalanine	4	1.0	263 ± 35	0.715	0.41 ± 0.08	0.242	19.04 ± 0.64	0.006
cholesterol sulfate	4	1.0	508 ± 169	0.040	0.99 ± 0.41	0.005	41.41 ± 7.73	<.001

TABLE 4

Compounds	N	Conc (%)	TEWL (T=4 hours)	P	Plasma Conc. (%dose /ml plasma)	P	Total Body Conc. (%)	P
conduritol-β-epoxide + fluindostatin	4	0.5/1.5	583 ± 128	0.001	0.84 ± 0.17	<.001	30.86 ± 6.06	<.001
conduritol-β-epoxide + TOFA	4	2.0/0.5	448 ±80	0.040	1.27 ± 0.44	0.001	42.44 ± 9.52	<.001
fumonisin B_1 + fluindostatin	4	0.5/1.5	533 ± 235	>.05	0.29 ± 0.09	<.006	11.58 ± 2.44	<.02
fumonisin B_1 + TOFA	4	0.5/0.5	613 ± 170	<.012	0.33 ± 0.08	<.003	10.02 ± 1.09	>.05
N-palmitoyl-DL-hydroxysphingosine + TOFA	3	0.5/0.5	148 ± 75	>.1	0.83 ± 0.25	<.0001	56.58 ± 14.48	<.0001
N-palmitoyl-DL-hydroxysphingosine + fluindostatin	4	0.5/1.5	650 ± 68	<.003	0.29 ± 0.04	<.0001	18.67 ± 1.38	<.0001
fumonisin B_1 + cholesterol sulfate	3	0.5/1.0	950 ± 43	<.0001	0.52 ± 0.03	<.0001	27.57 ± 6.93	<.001

TABLE 5

Compounds	N	Conc (%)	TEWL (T=4 hours)	P	Plasma Conc. (%dose /ml plasma)	P	Total Body Conc. (%)	P
conduritol-β-epoxide + bromophenacylbromide	4	0.5/0.05	398 ± 23	0.017	0.31 ± 0.04	0.968	16.73 ± 1.91	0.068
conduritol-β-epoxide + bromophenacylbromide	3	2.0/0.25	753 ± 149	<.001	1.60 ± 0.42	<.001	52.09 ± 6.28	<.001
desipramine + bromophenacylbromide	4	0.5/0.1	595 ± 89	<.001	0.64 ± 0.09	<.001	22.26 ± 3.99	0.002
desipramine + bromophenacylbromide	4	1.0/0.1	365 ± 113	0.233	0.43 ± 0.04	<.001	23.46 ± 1.69	<.001
fumonisin B_1 + bromophenacylbromide	4	0.5/0.15	710 ± 196	<.003	0.5 ± 0.16	<.0001	20.41 ± 5.11	<.0001
fumonisin B_1 + MJ33	3	0.5/1.5	573 ± 101	0.016	0.4 ± 0.03	<.0001	19.75 ± 0.83	<.0001
N-palmitoyl-DL-hydroxysphingosine + bromophenacylbromide	4	0.5/0.1	1000 ± 0	<.0001	0.47 ± 0.07	<.0001	33.24 ± 2.07	<.0001
bromophenacylbromide + deoxynojirimycin	4	0.2/4.0	753 ± 153	<.001	0.65 ± 0.11	<.001	34.60 ± 3.27	<.001

TABLE 6

Compounds	N	Conc (%)	TEWL (T=4 hours)	P	Plasma Conc. (%dose /ml plasma)	P	Total Body Conc. (%)	P
conduritol-β-epoxide (intact skin) - TID, total 7-8 doses	3	4.0	184 ± 39	0.008	0.82 ± 0.22	0.010	41.18 ± 10.69	0.010
conduritol-β-epoxide	4	4.0	253 ± 33	0.518	0.30 ± 0.04	0.809	15.70 ± 2.07	0.192
fumonisin B_1	3	0.5	440 ± 149	>.1	0.24 ± 0.03	<.003	14.58 ± 0.73	<.0001
N-palmitoyl-DL-hydroxysphingosine	4	0.5	793 ± 124	<.0001	0.21 ± 0.03	0.012	6.88 ± 0.69	>.1
N-oleoylethanolamine	4	0.5	720 ± 229	<.001	1.2 ± 0.32	<.0001	42.58 ± 2.4	<.0001
bromophenacylbromide (intact skin)	3	0.15	98 ± 47	0.127	0.19 ± 0.10	0.165	14.73 ± 5.86	0.092
bromophenacylbromide	4	0.2	690 ± 177	<.001	0.84 ± 0.07	<.001	39.42 ± 11.09	<.001

TABLE 7

Compounds	N	Conc (%)	TEWL (T=4 hours)	P	Plasma Conc. (%dose /ml plasma)	P	Total Body Conc. (%)	P
N-oleoylethanolamine + PDMP	4	0.5/0.1	368 ± 47	>0.1	0.9 ± 0.19	<.0001	41.77 ± 3.27	<.0001
N-oleoylethanolamine + 4-methylumbelliferyl-β-o-xyloside	4	0.5/1.0	525 ± 103	0.015	0.67 ± 0.06	<.0001	55.39 ± .9.44	<.0001
Acid lipase in MES pH 5.5 - tape stripping	4	1.0/20 mM	385 ± 32	0.002	0.75 ± 0.05	0.748	45.90 ± 4.47	0.027

TABLE 8

Compounds	Example	LHRH Delivery Ratio vs. Vehicle	SEM
TOFA + Lovastatin	8	1.9	0.7
TOFA + N-Palmitoyl-DL-dihydroxysphingosine	10	6.9	4.3
Bromphenacylbromide + N-Palmitoyl-DL-dihydroxysphingosine	11	7.6	1.5
Bromophenacylbreomide + Deoxynojirimycin	11	2.0	0.1
Fumonisin B_1 + MJ33	11	1.7	0.9
Fumonisin B_1	12	3.8	2.9
Acid Lipase	13	2.2	1.1

It will b apparent to on of ordinary skill in the art that many changes and modifications can be mad in the invention, now being fully described, without departing from the spirit or scope of th invention.

CLAIMS

1. A method for disrupting the epithelial barrier function in a host in need of the topical administration of a physiologically active substance, which comprises applying to the epithelium of the host, a barrier-disrupting amount of at least one agent selected from the group consisting of an inhibitor of ceramide synthesis, an inhibitor of glucosylceramide synthesis, an inhibitor of acylceramide synthesis, an inhibitor of sphingomyelin synthesis, an inhibitor of fatty acid synthesis, an inhibitor of cholesterol synthesis, inhibitors of phospholipid, glycosphingolipid, including glucosylceramide, acylceramide and sphingomyelin degradation, a degradation enzyme of free fatty acid, ceramide, sphingomyelin, acylceramide, or glucosylceramides and both inhibitors and stimulators of metabolic enzymes of free fatty acids, ceramide, and cholesterol.

2. The method according to claim 1, wherein the inhibitor of ceramide, acylceramide, sphingomyelin, or glucosylceramide synthesis is selected from the group consisting of inhibitors of serine palmitoyl transferase, inhibitors of ceramide synthetase, inhibitors of sphingomyelinase, inhibitors of β-glucosidase, inhibitors of acid lipase, inhibitors of α-hydroxylation, inhibitors of N-acyl chain length elongation, inhibitors of ω-acyl transferases, inhibitors of glucosyl transferase, and inhibitors of phosphatidylcholine-ceramide phosphorylcholine transferase.

3. The method according to claim 1, wherein the stimulator of ceramide metabolism distal to ceramide is selected from the group consisting of ω-acyl transferase, glucosyltransferase, and phosphatidylcholine-ceramide phosphorylcholine transferase.

4. The method according to claim 2, wherein the inhibitor of serine palmitoyl transferase is selected from the group consisting of D-cycloserine, β-chlorolanine, fluoropalmitate, L-cycloserine, and β-fluoroalanine.

5. The method according to claim 2, wherein the inhibitor of ceramide synthetase is a fumonisin.

6. The method according to claim 2, wherein the inhibitor of sphingomyelinase is selected from the group consisting of tricyclodecan-9yl-xanthogenate, an aminoglycoside, ethylisopropylamiloride, a tricyclic, a phenothiazine, N-palmitoyl-DL-dihydroxysphingosine and methylene-dioxybenzapine.

7. The method according to claim 6, wherein the aminoglycoside is gentamicin or neomycin.

8. The method according to claim 6, wherein the tricyclic is despramine or imipramine.

9. The method according to claim 6, wherein the phenothiazine is chlorpromazine or perchlorperazine.

10. The method according to claim 2, wherein the inhibitor of β-glucosidase is selected from the group consisting of N-hexylglucosyl-sphingosine, bromoconduritol-B-epoxide, conduritol, cyclophellitol, conduritol-B-epoxide, and deoxynojirimycin.

11. The method according to claim 2, wherein the inhibitor of acid lipase is selected from the group consisting of boronic acid, phenylboronic acid, tetrahydrolipstatin and esterasin.

12. The method according to claim 2, wherein the inhibitor of glucosyl transferase is selected from the group consisting of 1-phenyl-2-decanoylamine-3-morpholine-1-propanol (PDMP), its analogs, including PPMP, p-nitro-phenyl-α-xyloside, 4-methyl umbelliferyl-β-O-xyloside, and O-and p-nitrophenyl-β-O-xylopyaranoside and the inhibitor of ceramidase is N-oleoyl-ethanolamine.

13. The method according to claim 1, wherein the inhibitor of free fatty acid synthesis is selected from the group consisting of inhibitors of acetyl CoA carboxylase, inhibitors of fatty acid synthetase, and inhibitors of phospholipase.

14. The method according to claim 10, wherein the inhibitor of acetyl CoA carboxylase is 5-tetradecyloxy-2-furancarboxylic acid (TOFA).

15. The method according to claim 10, wherein the inhibitor of phospholipase is selected from the group consisting of gomisin A, 2-(p-amylcinnamyl) amino-4-chlorobenzoic acid, bromophenacylbromide, monoalide, 7,7-dimethyl-5,8-eicosadienoic acid, nicergoline, cepharanthine, quercetin, dibutyryl-cyclic AMP, diaminoethoxyhexesterol, N-oleoylethanolamine, N-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphostidylserine, cyclosporine A, a topical anesthetic such as dibucaine, prenylamine, retinoids, such as all-trans and 13-cis-retinoic acid, W-7, phenothiazines such as trifluoperazine, R-24571 (calmidazolium), 1-hexadecyl-3-trifluoroethyl glycero-sn-2-phosphomenthol (MJ33), calcium channel blockers, such as verapamil,

diltiazem, nifedipine, nimodipine, antimalarials such as quinacrine, mepacrine, chloroquine hydroxychloroquine, beta blockers such as propanalol, labetalol, calmodulin antagonists, EGTA, thimersol, glucocorticosteroids such as dexamethasone, prednisolone, and nonsteroidal antiinflammatory agents such as indomethacin and naproxen.

16. The method according to claim 13, wherein the stimulator of fatty acid metabolism is an enzyme selected from the group comprising the fatty acid to phospholipid metabolic pathway.

17. The method according to claim 1, wherein the inhibitor of cholesterol synthesis is selected from the group consisting of inhibitors of HMG CoA reductase, squalene epoxidase, squalene synthetase, cholesterol sulfate, phosphate synthetase, and $\Delta 7$ or $\Delta 24$ reductase.

18. The method according to claim 1, wherein the stimulator of cholesterol metabolism distal to cholesterol is a synthetic enzyme of a steroid hormone.

19. The method according to claim 1, wherein epithelial barrier is disrupted using two barrier disrupting agents from different groups.

20. The method according to claim 16, wherein the inhibitor of HMG CoA reductase is selected from the group consisting of simvastatin, lovastatin, fluindostatin, pravastatin, mevastatin, cholesterol sulfate, cholesterol phosphate, and 25-OH or 26-OH cholesterol.

21. The method according to claim 16, wherein the inhibitor of $\Delta 7$, $\Delta 24$ reductase is selected from the group consisting of 22,25-diazacholesterol, 20,25-diazacholesterol, AY9944 and triparanol.

22. The method according to claim 1, wherein the inhibitor of glycosphingolipid degradation is selected from the group consisting of bromoconduritol-B-epoxide; conduritol-B-epoxide, and cyclophellitol.

23. The method according to claim 1, wherein the degradation enzyme of ceramide is ceramidase.

24. The method according to claim 1, wherein the degradation enzymes of acylceramide are acid lipase and ceramidase.

25. The method according to claim 1, wherein the degradation enzymes of glucosylceramide are β-glucosidase and ceramidase, and the degradation enzyme of sphingomyelin is sphingomyelinase.

26. The method according to claim 1, wherein the inhibitor of ceramide synthesis, acylceramide synthesis, glycosylceramide synthesis, sphingomyelin synthesis, free fatty acid synthesis, cholesterol synthesis, is present at a concentration of from about 0.01% to about 5.0% by weight of the total.

27. The method according to claim 1, wherein the composition is a lotion, cream, ointment, solution, paste, suppository, aerosol, nebulized formulation, or gel.

28. The method according to claim 1, wherein the composition further contains a known epithelial penetration enhancer.

29. The method according to claim 26, wherein the penetration enhancer is selected from the group consisting of 1-dodecylazacycloheptan-2-on , DMSO, propylene glycol, oleyl alcohol, and methyl pyrrolidone.

30. The method according to claim 1, wherein the composition furth r contains an effective amount of a physiologically active substance.

31. A topical composition for disrupting the epithelial barrier function in a host in need of topical administration of a physiologically active substance, which comprises an epithelial barrier disrupting amount of at least one agent selected from the group consisting of an inhibitor of ceramide synthesis, an inhibitor of acylceramide synthesis, an inhibitor of glucocylceramide synthesis, an inhibitor of sphingomyelin synthesis, an inhibitor of fatty acid synthesis, an inhibitor of cholesterol synthesis, inhibitors of phospholipid, glycosphingolipid, including glucosylceramide, acylceramide and sphingomyelin degradation, a degradation enzyme of free fatty acid, ceramide, sphingomyelin, acylceramide, or glucosyl-ceramides and both inhibitors and stimulators of metabolic enzymes of free fatty acids, ceramide, and cholesterol, together with a pharma-ceutically acceptable carrier.

32. The composition according to claim 31, wherein the active substance is present at a concentration of about 0.001% to about 60% by weight of the total.

33. The composition according to claim 31, wherein the inhibitor of ceramide, acylceramide, sphingomyelin or glucosylceramide synthesis is selected from the group consisting of inhibitors of serine palmitoyl transferase, inhibitors of ceramide synthetase, inhibitors of sphingomyelinase, inhibitors of β-glucosidase, inhibitors of phospholipase, inhibitors of acid lipase, inhibitors of ω-acyl transferases, inhibitors of glucosyl transferase, and inhibitors of phosphatidylcholine-ceramide phosphorylcholine transferase.

34. The composition according to claim 31, wherein the stimulator of ceramide metabolism distal to ceramide is selected from the group consisting of ω-acyl transferase, glucosyltransferase, and phosphatidylcholine-ceramide phosphorylcholine transferase.

35. The composition according to claim 31, wherein the inhibitor of serine palmitoyl transferase is selected from the group consisting of D-cycloserine, β-chlorolanine, fluoropalmitate, L-cycloserine, and β-fluoroalanine.

36. The composition according to claim 31, wherein the inhibitor of ceramide synthetase is a fumonisin.

37. The composition according to claim 31, wherein the inhibitor of sphingomyelinase is selected from the group consisting of tricyclodecan-9yl-xanthogenate, an aminoglycoside, ethylisopropylamiloride, a tricyclic, a phenothiazine, N-palmitoyl-DL-dihydroxysphingosine and methylene-dioxybenzapine.

38. The composition according to claim 37, wherein the aminoglycoside is gentamicin or neomycin.

39. The composition according to claim 37, wherein the tricyclic is despramin or imipramine.

40. The composition according to claim 37, wherein the phenothiazine is chlorpromazine, perchlorperazine, or trifluoperazine.

41. The composition according to claim 31, wherein the inhibitor of β-glucosidase is selected from the group consisting of N-hexylglucosyl-sphingosine, bromoconduritol-B-epoxide, conduritol, cyclophellitol, conduritol-B-epoxide, and deoxynojirimycin.

42. The composition according to claim 31, wherein the inhibitor of acid lipase is selected from the group consisting of boronic acid, phenyl-boronic acid, tetrahydrolipstatin and esterasin.

43. The composition according to claim 31, wherein the inhibitor of glucosyl transferase is selected from the group consisting of 1-phenyl-2-decanoylamine-3-morpholine-1-propanol (PDMP), its analogs, including PPMP, p-nitro-phenyl-α-xyloside, 4-methyl umbelliferyl-β-O-xyloside, and O-and p-nitrophenyl-β-O-xylopyaranoside, and the inhibitor of ceramidase is N-oleoyl-ethanolamine.

44. The composition according to claim 31, wherein the inhibitor of free fatty acid synthesis is selected from the group consisting of inhibitors of acetyl CoA carboxylase, inhibitors of fatty acid synthetase, and inhibitors of phospholipase.

45. The composition according to claim 39, wherein the inhibitor of acetyl CoA carboxylase is 5-tetradecyloxy-2-furancarboxylic acid (TOFA).

46. The composition according to claim 44, wher in the inhibitor of phospholipase is selected from the group consisting of gomisin A, 2-(p-amylcinnamyl) amino-4-chlorobenzoic acid, bromophenacylbromid , monoalide, 7,7-dimethyl-5,8-eicosadienoic acid, nicergoline, cepharanthine, quercetin, dibutyryl-cyclic AMP, diaminoethoxyhexesterol, N-oleoylethanolamine, N-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphostidylserine, cyclosporine A, a topical anesthetic such as dibucaine, prenylamine, retinoids, such as all-trans and 13-cis-retinoic acid, W-7, phenothiazines such as trifluoperazine, R-24571 (calmidazolium), 1-hexadecyl-3-trifluoroethyl glycero-sn-2-phosphomenthol (MJ33), calcium channel blockers, such as verapamil, diltiazem, nifedipine, nimodipine, antimalarials such as quinacrine, mepacrine, chloroquine hydroxychloroquine, beta blockers such as propanalol, labetalol, calmodulin antagonists, EGTA, thimersol, glucocorticosteroids such as dexamethasone, prednisolone, and nonsteroidal antiinflammatory agents such as indomethacin and naproxen.

47. The composition according to claim 31, wherein the inhibitor of cholesterol synthesis is selected from the group consisting of inhibitors of HMG CoA reductase, squalene epoxidase, squalene synthetase, cholesterol sulfate, phosphate synthetase, and $\Delta 7$ or $\Delta 24$ reductase.

48. The composition according to claim 31, wherein the stimulator of cholesterol metabolism distal to cholesterol is a synthetic enzyme of steroid hormone.

49. Th composition according to claim 47, wherein the inhibitor of HMG CoA reductase is selected from the group consisting of simvastatin, lovastatin, fluindostatin, pravastatin, mevastatin, cholesterol sulfate, cholesterol phosphate, and 25-OH or 26-OH cholesterol.

50. The composition according to claim 47, wherein the inhibitor of $\Delta 7$, $\Delta 24$ reductase is selected from the group consisting of 22,25-diazacholesterol, 20,25-diazacholesterol, AY9944 and triparanol.

51. The composition according to claim 31, wherein the inhibitor of phospholipase is selected from the group consisting of gomisin A, 2-(p-amylcinnamyl) amino-4-chlorobenzoic acid, bromophenacylbromide, monoalide, 7,7-dimethyl-5,8-eicosadienoic acid, nicergoline, cepharanthine, quercetin, dibutyryl-cyclic AMP, diaminoethoxyhexesterol, N-oleoylethanolamine, N-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphostidylserine, cyclosporine A, a topical anesthetic such as dibucaine, prenylamine, retinoids, such as all-trans and 13-cis-retinoic acid, W-7, phenothiazines such as trifluoperazine, R-24571 (calmidazolium), 1-hexadecyl-3-trifluoroethyl glycero-sn-2-phosphomenthol (MJ33), calcium channel blockers, such as verapamil, diltiazem, nifedipine, nimodipine, antimalarials such as quinacrine, mepacrine, chloroquine hydroxychloroquine, beta blockers such as propanalol, labetalol, calmodulin antagonists, EGTA, thimersol, glucocorticosteroids such as dexamethasone, prednisolone, and nonsteroidal antiinflammatory agents such as indomethacin and naproxen.

52. The composition of according to claim 44, wherein the stimulator of fatty acid metabolism is an enzyme selected from the group comprising the fatty acid to phospholipid metabolic pathway.

53. The composition according to claim 31, wh rein the degradation enzyme of ceramide is ceramidas .

54. The composition according to claim 31, wherein the degradation enzymes of acylceramide are acid lipase and ceramidase.

55. The composition according to claim 31, wherein the degradation enzymes of glucosylceramide are β-glucosidase and ceramidase, and the enzyme of sphingomyelin is sphingomyelinase.

56. The composition according to claim 31, wherein the inhibitor of ceramide synthesis, free fatty acid synthesis, cholesterol synthesis, acylceramide synthesis, sphingomyelin synthesis and glucosylceramide synthesis, if present, is present at a concentration of from about 0.01% to about 5.0% by weight of the total.

57. The composition according to claim 31, wherein the composition is a lotion, cream, ointment, solution, paste, suppository, aerosol, nebulized formulation, or gel.

58. The composition according to claim 31, further comprising an epithelial penetration enhancing compound.

59. The composition according to claim 56, wherein the epithelial penetration enhancing compound is selected from the group consisting of 1-dodecylazacycloheptan-2-one, DMSO, propylene glycol, oleyl alcohol, and methyl pyrrolidone.

60. The composition according to claim 31, further comprising a therapeutically effective amount of the physiologically active substance.

61. A topical composition comprising:

(a) 0% to about 5.0% by weight of an inhibitor of ceramide synthesis;

(b) 0% to about 5.0% by weight of an inhibitor of free fatty acid synthesis;

(c) 0% to about 5.0% by weight of an inhibitor of cholesterol synthesis;

(d) 0% to about 5.0% by weight of an inhibitor of degradation selected from the group consisting of phospholipid, glycosphingolipid, including glucosylceramide, sphingomyelin, and acylceramide ;

(e) 0% to about 5.0% by weight of a degradation enzyme for ceramide or free fatty acid;

(f) 0% to about 5.0% by weight of stimulators of steps of metabolism of ceramide, free fatty acid and cholesterol metabolism distal to ceramide, free fatty acid, and cholesterol, respectively; and

(g) a sufficient amount of a physiologically acceptable carrier to total 100%.

62. The composition according to claim 55, further comprising about 0.001% to about 20% by weight of a physiologically active substance.

63. The composition according to claim 62, wherein the physiologically active substance is selected from the group consisting of an antimicrobial, anti-inflammatory, anti-oxidant, antineoplastic, antiarrhythmic, anesthetic, cytokine and other biological response modifiers, antihistamine, antiepileptic, antihypertensive, analgesic, antiandrogen, vasodilator, antitussive, neuroleptic, peptides, substance P, capsaicin, enzymes, hormonal, and nutritional agent.

64. The composition according to claim 62, wherein the physiologically active substance is selected from the group consisting of lidocaine, LHRH, caffeine, and vasopressin.

65. The composition according to claim 61, wherein the inhibitor of ceramide, acylceramide, sphingomyelin, or glucosylceramide synthesis is selected from the group consisting of inhibitors of serine palmitoyl transferase, inhibitors of ceramide synthetase, inhibitors of sphingomyelinase, inhibitors of β-glucosidase, inhibitors of acid lipase, inhibitors of omega-acyl transferases, inhibitors of glucosyl transferase, and inhibitors of phosphatidylcholine-ceramide phosphorylcholine transferase.

66. The composition according to claim 61, wherein the stimulator of ceramide metabolism distal to ceramide is selected from the group consisting of ω-acyl transferase, glucosyltransferase, and phosphatidylcholine-ceramide phosphorylcholine transferase.

67. The composition according to claim 61, wherein the inhibitor of free fatty acid synthesis is selected from the group consisting of inhibitors of acetyl CoA carboxylase, inhibitors of fatty acid synthetase, and inhibitors of phospholipase.

68. The composition according to claim 61, wherein the inhibitor of cholesterol synthesis is selected from the group consisting of inhibitors of HMG CoA reductase, squalene epoxidase, squalene synthetase, as well as oxygenated sterols, cholesterol sulfate or phosphate, and $\Delta 7$ or $\Delta 24$ reductase.

69. The composition according to claim 61, wherein the stimulator of cholesterol metabolism distal to cholesterol is a synthetic enzyme of a steroid hormone.

70. The composition according to claim 61, further comprising an epithelial penetration enhancing compound.

71. The composition according to claim 70, wherein the epithelial penetration enhancing compound is selected from the group consisting of 1-dodecylazacycloheptan-2-one, DMSO, propylene glycol, oleyl alcohol, and methyl pyrrolidone.

72. The composition according to claim 63, wherein the composition comprises at least two critical lipid synthetic enzyme inhibitors.

73. The composition according to claim 72, wherein the two inhibitors are selected from the group consisting of TOFA, β-chloroalanine or L-cycloserine, cholesterol sulfate, fluindostatin, lovastatin, fumosin B1, N-palmitoyl-DL-dihydroxy-sphingosine, chonduritol epoxide, desipramine, bromophenyl bromide, MJ33, deoxynojirimycin, PDMP, N-oleoylethanolamine, 4-methylumbelliferyl-β-o-xyloside, and acid lipase.

74. The composition according to claim 72, wherein one inhibitor is selected from the group consisting of TOFA, β-chloroalanine or L-cycloserine, cholesterol sulfate, fluindostatin, lovastatin, fumosin B1, N-palmitoyl-DL-dihydroxy-sphingosine, chonduritol epoxide, desipramine, bromophenyl bromide, MJ33, deoxynojirimycin, PDMP, N-oleoylethanolamine, 4-methylumbelliferyl-β-o-xyloside, and acid lipase.

75. The composition according to claim 72, wherein th two inhibitors are TOFA and betachloroalanin and wherein th activ agent is lidocaine.

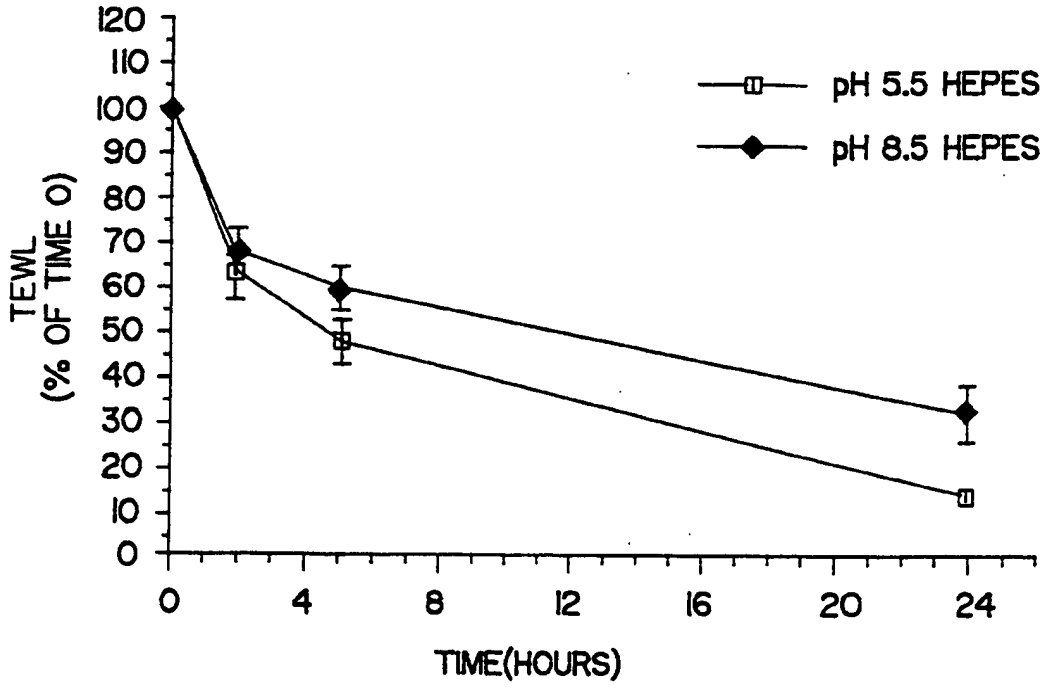

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70
60
50
40
30
20
10
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TEWL
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12
16
20
24
TIME(HOURS)
pH 5.5 HEPES
pH 8.5 HEPES

FIG. 1

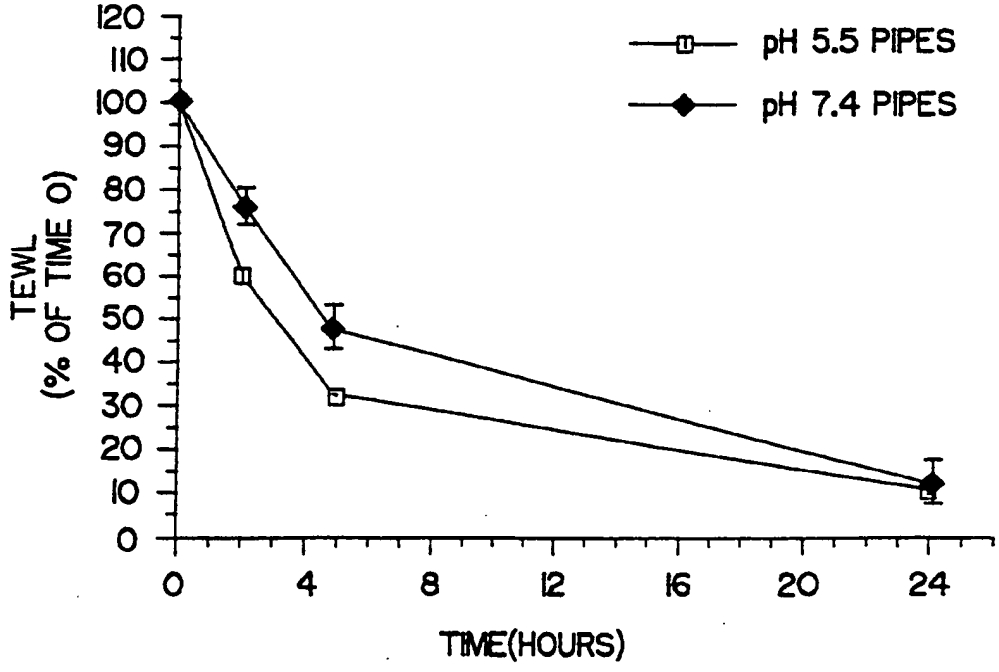

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TEWL
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16
20
24
TIME(HOURS)
pH 5.5 PIPES
pH 7.4 PIPES

FIG. 2

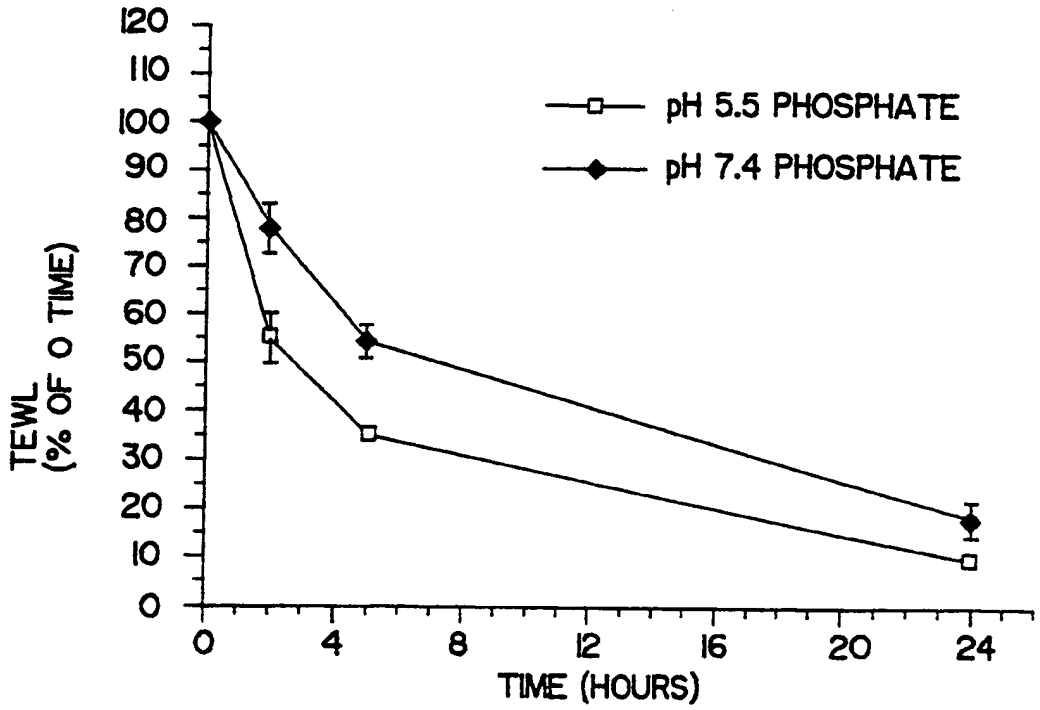

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30
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10
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TEWL
(% OF 0 TIME)
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12
16
20
24
TIME (HOURS)
pH 5.5 PHOSPHATE
pH 7.4 PHOSPHATE

FIG. 3

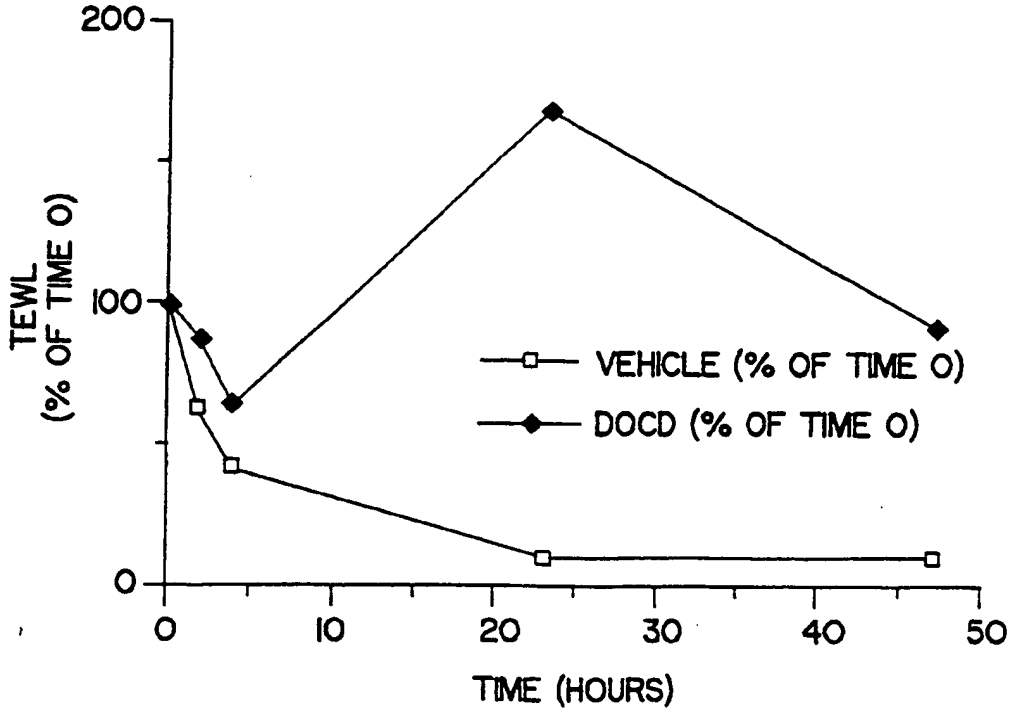

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TEWL
(% OF TIME 0)
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30
40
50
TIME (HOURS)
VEHICLE (% OF TIME 0)
DOCD (% OF TIME 0)

FIG. 4

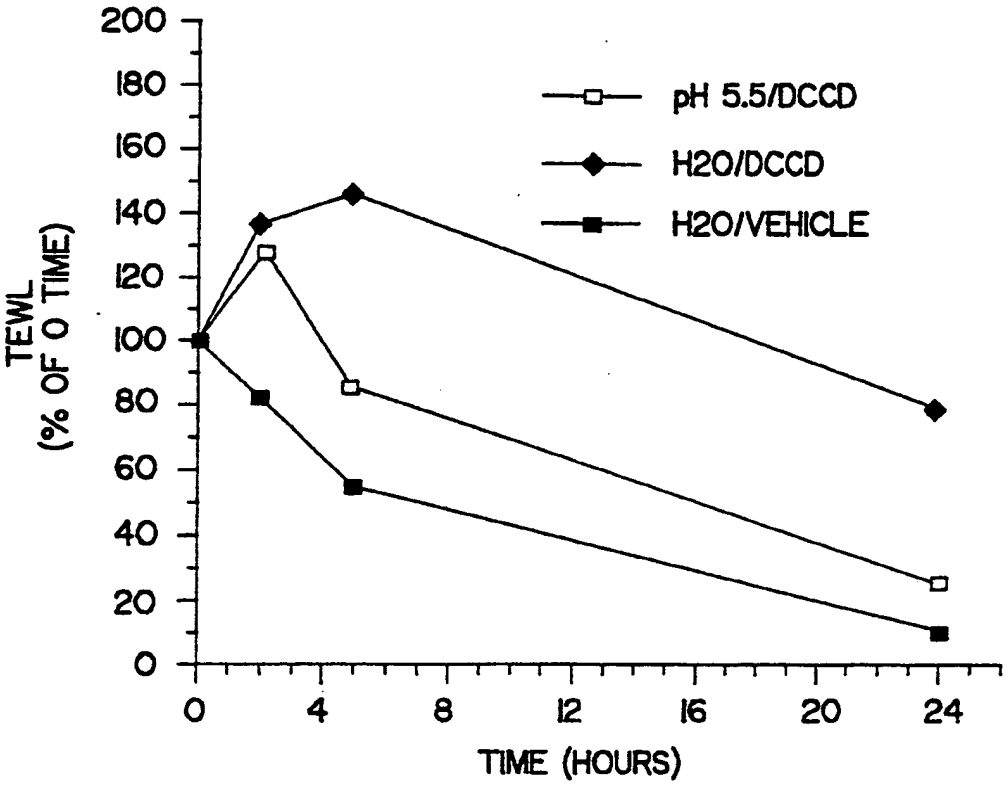

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140
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100
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60
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0
TEWL
(% OF 0 TIME)
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4
8
12
16
20
24
TIME (HOURS)
pH 5.5/DCCD
H2O/DCCD
H2O/VEHICLE

FIG. 5

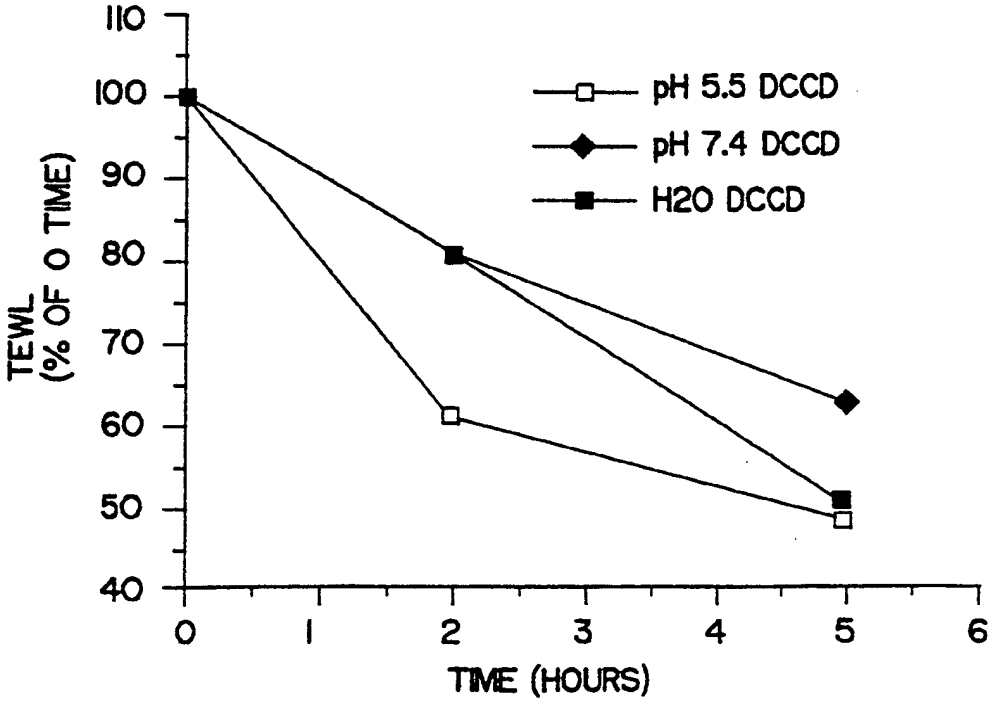

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TEWL
(% OF 0 TIME)
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4
5
6
TIME (HOURS)
pH 5.5 DCCD
pH 7.4 DCCD
H2O DCCD

FIG. 6

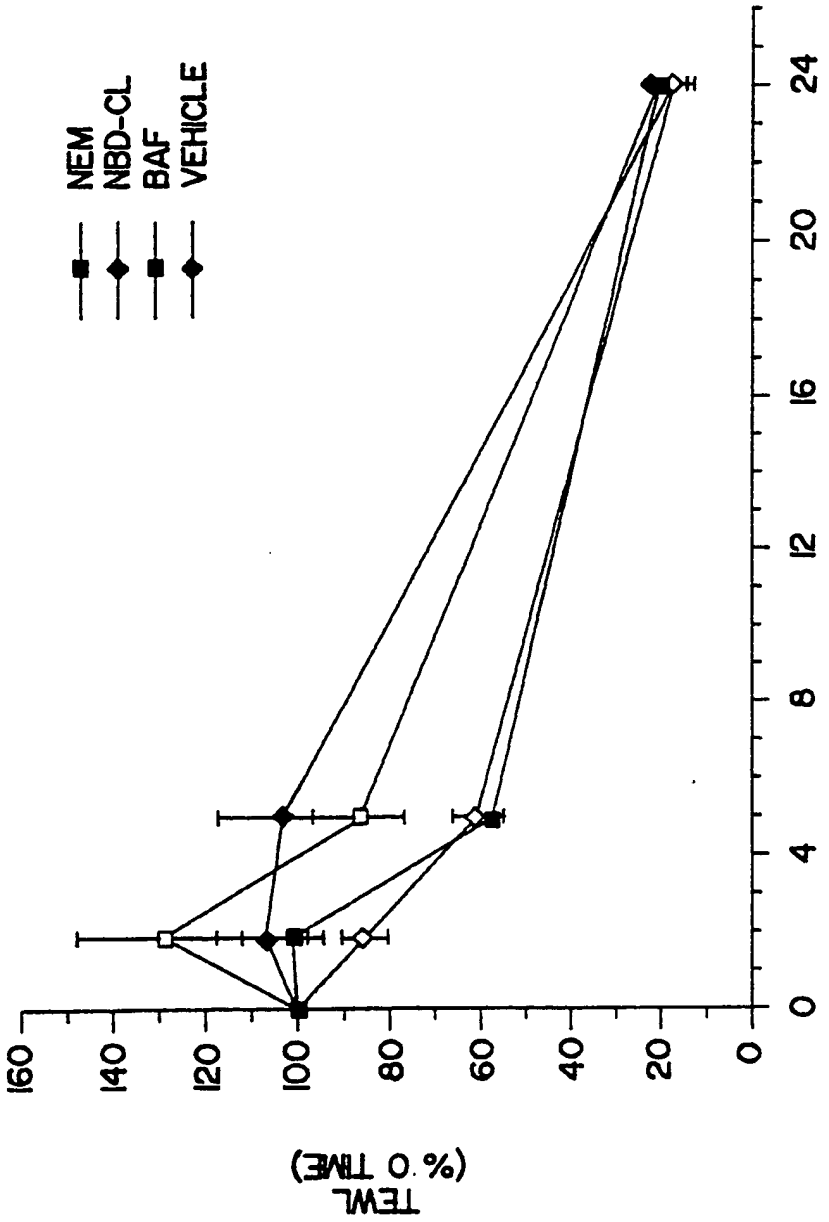

NEM
NBD-CL
BAF
VEHICLE
TEWL (% 0 TIME)
160
140
120
100
80
60
40
20
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4
8
12
16
20
24
TIME (HOURS)

FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03030

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 9/10
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/43; 514/937

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,177,267 (HERSCHLER) 04 December 1979, see entire document.	1-75
Y, P	US, A, 5,215,759 (MAUSNER) 01 June 1993, see entire document.	1-75
Y	US, A, 5,073,372 (TURNER ET AL.) 17 December 1991, see entire document.	1-75

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02 JUNE 1994	JUN 29 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RAJ BAWA Telephone No. (703) 308-2351

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03030

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/43